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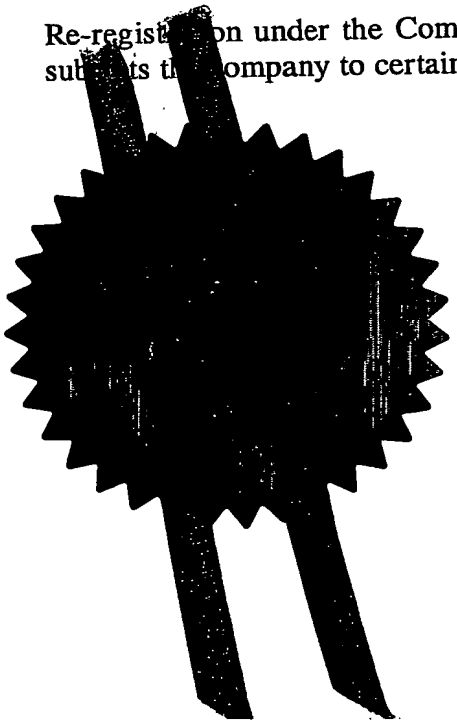

Signed R. Mahoney

Dated 30 October 2003

Patents Form 1/77

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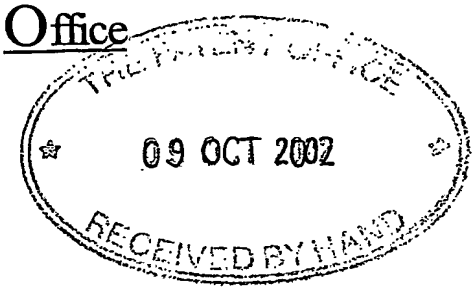

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1. Your reference GBP86463

2. Patent application number (The Patent Office will fill in this part) 09 OCT 2002 0223494.6

3 Full name, address and postcode of the or of each applicant (underline all surnames)

Neuropharma, S.A.,
Jose Abascal 2
28003 Madrid
Spain

Patents ADP number (if you know it) 08481707001

If the applicant is a corporate body, give the country/state of its incorporation Spain

4. Title of the invention DUAL BINDING SITE ACETYLCHOLINESTERASE INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

5. Name of your agent (if you have one) "Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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Country	Priority application No (if you know it)	Date of filing (day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

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DUPLICATE

Document : 865588

DUAL BINDING SITE ACETYLCHOLINESTERASE INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

FIELD OF THE INVENTION

The invention relates to the synthesis and biological evaluation of dual site acetylcholinesterase inhibitors, specially to a series of tacrine derivatives useful for the treatment of Alzheimer's disease.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is one of the most common causes of mental deterioration in elderly people, accounting for about 50-60 % of the overall cases of dementia among persons over 65 years of age [1]. Demographic data indicate that the percentage of elderly in the population is increasing.

Brain regions that are associated with higher mental functions, particularly the neocortex and hippocampus, are those most affected by the characteristic pathology of AD [2]. This includes the extracellular deposits of β-amyloid (derived from amyloid precursor protein, APP) in senile plaques [3, 4], intracellular formation of neurofibrillary tangles (containing an abnormally phosphorylated form of a microtubule associated protein, tau) [5, 6], and the loss of neuronal synapsis and pyramidal neurons [7].

The past two decades have witnessed a considerable research effort directed towards discovering the cause of AD with the ultimate hope of developing safe and effective pharmacological treatments [8]. Nowadays, research in the knowledge of the pathogenic cascade that characterizes AD has provided a robust framework for new therapeutic intervention targets [9].

Nevertheless, current treatment approaches in this disease continue being primarily symptomatic [10], with the major therapeutic strategy based on the cholinergic hypothesis [11] and specifically on acetylcholinesterase (AChE) inhibition. The successful development of these compounds was based on a well-accepted theory that the decline in cognitive and mental functions associated with AD is related to the loss of cortical cholinergic neurotransmission [12]. This link between cholinergic dysfunction in the basal-cortical system and cognitive deficits has focused scientific efforts on developing tools to elucidate

the neurobiological role of the cholinergic system in cognition and to elucidate therapeutic interventions in the disorder [13]. As result, over last decade, the cholinergic hypothesis of AD has launched on the market various cholinergic drugs primarily AChE inhibitors as tacrine [14], donepezil [15] or rivastigmine [16], and more recently galanthamine [17], fig. (1), indicated modest improvement in the cognitive function of Alzheimer's patients.

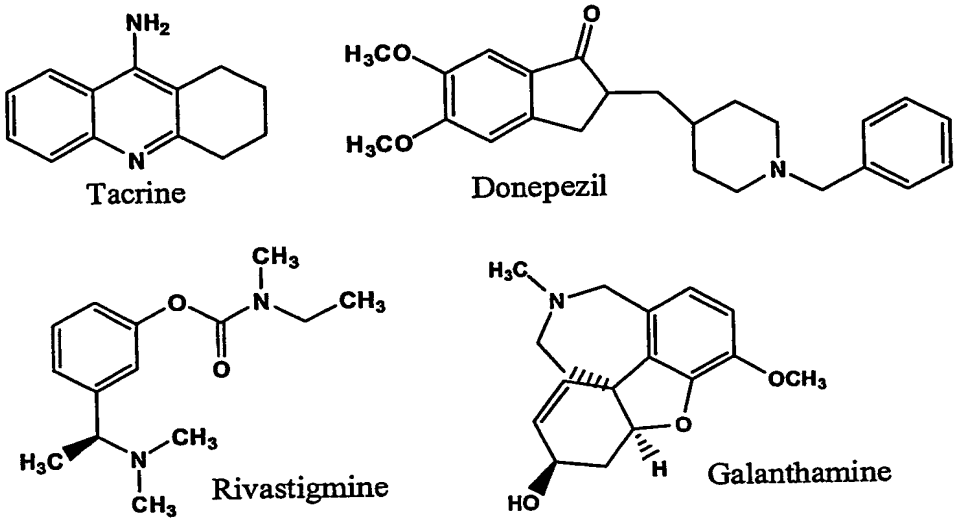


Figure 1

The three dimensional structure of AChE, as determined by x-ray crystallography, revealed that its active site can apparently be reached only through a deep and narrow "catalytic gorge" [18]. Inhibitors of AChE act on two target sites on the enzyme, the active site and the peripheral site. Inhibitors directed to the active site prevent the binding of a substrate molecule, or its hydrolysis, either by occupying the site with a high affinity molecule (tacrine) [19] or by reacting irreversibly with the catalytic serine (organophosphates and carbamates) [20]. The peripheral site consists of a less well-defined area, located at the entrance of the catalytic gorge. Inhibitors that bind to that site include small molecules, such as propidium [21, 22] and peptide toxins as fasciculins [23]. Bis-quaternary inhibitors as decamethonium [24], simultaneously bind to the active and peripheral sites, thus occupying the entire catalytic gorge.

Parallel to the development of antidementia drugs, research efforts have been focused, among others, on the therapeutic potential of AChE inhibitors to slow the disorder progression. This fact was based on a range of evidence, which showed that AChE has secondary non-cholinergic functions [25, 26].

New evidence shows that AChE may have a direct role in neuronal differentiation [27]. Transient expression of AChE in the brain during embryogenesis suggests that AChE may function in the regulation of neurite outgrowth [28, 29] and in the development of axon tracts [30]. Additionally, the role of AChE in cell adhesion have been studied [31]. The results indicate that AChE promotes neurite outgrowth in neuroblastoma cell line through a cell adhesive role [32]. Moreover, recent studies have shown that the peripheral anionic site of the AChE is involved in the neurotrophic activity of the enzyme [33] and conclude that the adhesion function of AChE is located at the peripheral anionic site [34]. This finding has implications, not only for our understanding of neural development and its disorders, but also for the treatment of neuroblastoma, the leukemias, and especially for Alzheimer's disease [35].

As it has been previously mentioned, senile plaques are one of pathological hallmarks in AD in which their main component is βA peptide. This is found as an aggregated poorly soluble form. In contrast soluble βA is identified normally circulating in human body fluids. Structural studies of βA showed that synthetic peptides containing the sequences 1-40 and 1-42 of βA can adopt two major conformational states in solution: an amyloidogenic conformer (βA ac) with a high content of β-sheet and partly resistant to proteases and a non-amyloidogenic conformer (βA nac) with a random coil conformation or α-helix and protease-sensitive. AChE colocalizes with βA peptide deposits present in the brain of Alzheimer's patients [36]. It is postulated that AChE binds to a βA nac form acting as a "pathological chaperone" and inducing a conformational transition from βA nac into βA ac in vitro and therefore to amyloid fibrils [37]. AChE directly promotes the assembly of βA peptide into amyloid fibrils forming stable βA-AChE complexes [38]. These complexes are able to change the biochemical and pharmacological properties of the enzyme and cause an increase in the neurotoxicity of the βA fibrils [39, 40]. Moreover, the interaction between these two molecules to form the complex was confirmed by crosslinking experiments [41]. Different studies concerned to the establishment of the binding site of AChE on Aβ have suggested that hydrophobic interactions may play a role in the stabilization of the βA-AChE complex probably due to specific binding to peripheral sites [42].

Considering the non-cholinergic aspects of the cholinergic enzyme AChE, their relationship to Alzheimer's hallmarks and the role of the peripheral site of AChE in all these functions, an attractive target for the design of new antidementia drugs emerged. **Peripheral or dual site inhibitors of AChE may simultaneously alleviate the cognitive deficit** in

Alzheimer's patients **and** what it is more important, **avoid the assembly of beta-amyloid** which represents a new way to delay the neurodegenerative process.

As revealed by the crystallographic structure of AChE and their inhibitors complexes, the AChE active site contains a catalytic triad (Ser 200, His 440, Glu 327) located at the bottom of a deep and narrow gorge, lined with aromatic residues and a subsite, including Trp 84, located near the bottom of the cavity. Trp 84 has been identified as the binding site of the quaternary group of acetylcholine, decamethonium and edrophonium [18]. In addition, Trp 279 at the peripheral site, located at the opening of the gorge, is involved in the binding of the second quaternary group of decamethonium being responsible for the adhesion function of the enzyme [34].

These residues **(Trp 84 and 279) have been the basis of the design** of a new generation of AChE inhibitors. Thus, ligands able to interact simultaneously with active and peripheral sites could implicate several advantages over the known inhibitors. On one hand, they should improve greatly the inhibitory potency and on the other hand they should be involved in neurotrophic activity (fig. 2).

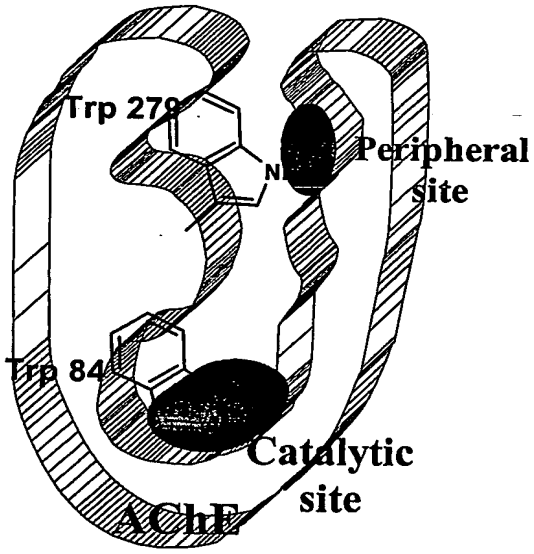
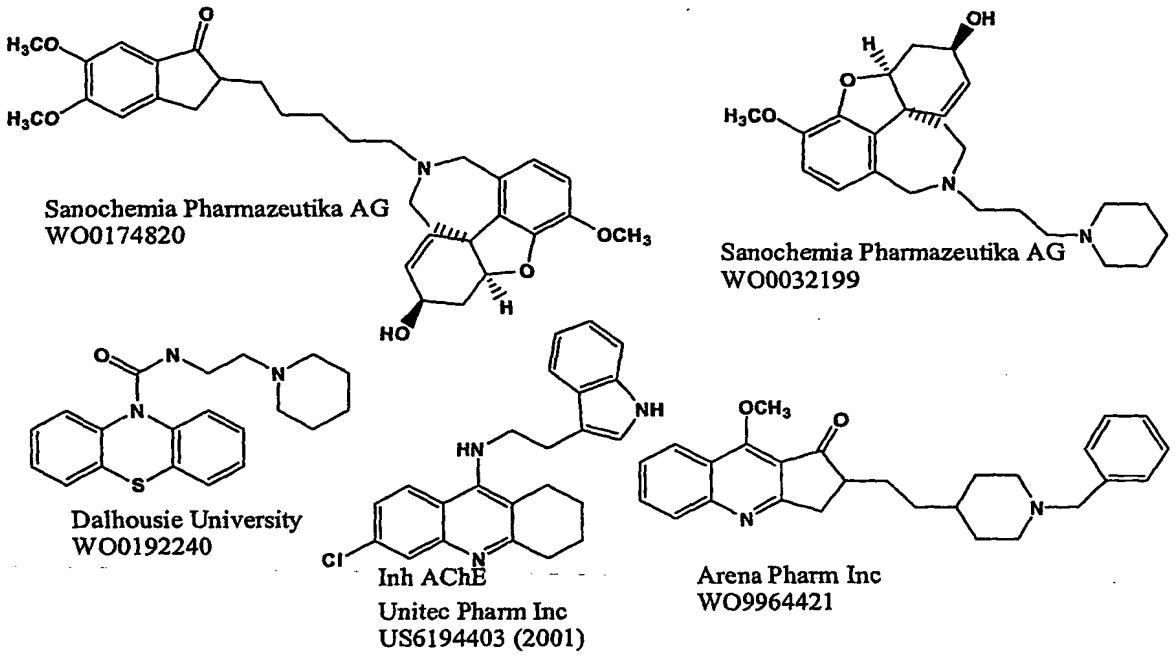


Figure 2

Here, we want to claim this new concept for the design of dual AchE inhibitors, eg compounds that, following this idea, show potent AchE inhibition activities together with modifications in the β-amyloid aggregation properties.

A review of derivatives that regarding theii chemical structure could be classified as dual AchE inhibitors has been described previously by us [43]. One of the compounds therein reported, a bis-galanthamine inhibitor, is recently described by molecular modelling techniques as a bis-functional ligand for the AchE [44].

Moreover, in fig 3 derivatives that could be also classified as dual AchE inhibitors are collected:



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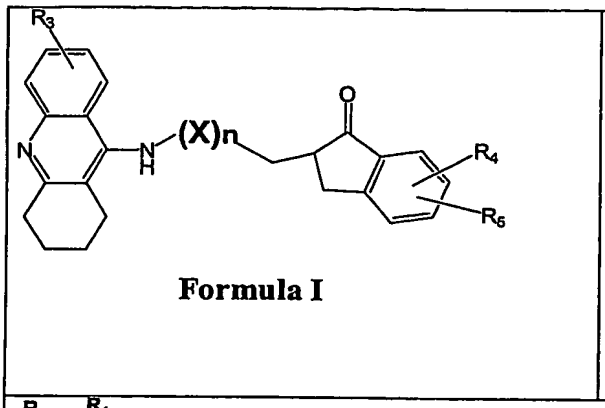
BRIEF DESCRIPTION

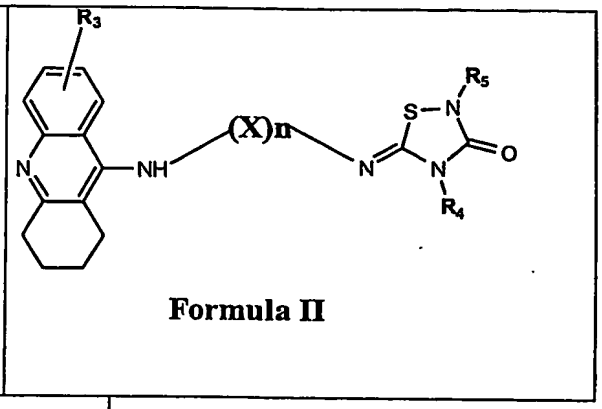
The invention discloses a series of acridine related derivatives for the treatment of Alzheimer's disease. Examples comprise bis-functional compounds as thiadiazolidinones-tacrine, indanone-tacrine or bis-acridine derivatives. Based on the above references, these acridine related derivatives should have very good potential for treating Alzheimer's disease, cognitive disorders and/or neurodegenerative dementing disease with aberrant protein aggregations.

DETAILED DESCRIPTION

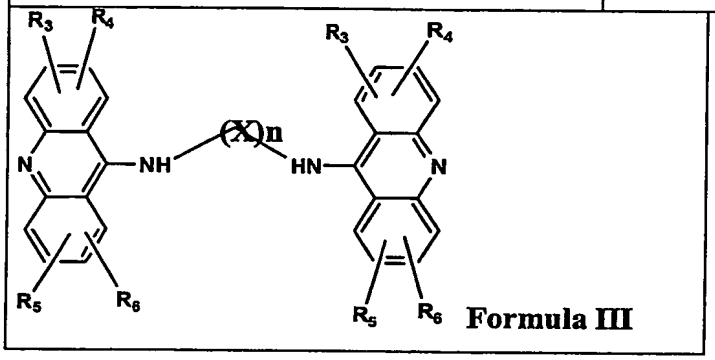
The invention is directed to the compounds represented by the general formula I, II and III:



Formula I



Formula II



Formula III

Where:

X is $-C(R^1)(R^2)-$, -CO-, -O- or $-NR^1-$

n is zero, one, two, three, four, five, six, seven, eight, nine or ten

R^1 and R^2 are independently selected from hydrogen, alkyl, aryl, halo, haloalkyl

R^3, R^4, R^5 and R^6 are independently selected from hydrogen, alkyl, cycloaklyl, haloalkyl, halo, aryl, $-(Z)_n$-aryl, heteroaryl, $-OR^3$, $-C(O)R^3$, $-C(O)OR^3$, $-S(O)_t-$

t is zero, one or two

Z is independently selected from $C(R^3)(R^4)-$, -C(O)-, -O-, $-C(=NR^3)-$, $-S(O)_t-$, $N(R^3)-$

Definitions

Unless otherwise specified, the following terms have the following meaning:;

"alkyl" refers to a straight-line or branched hydrocarbon chain comprising only atoms of carbon and hydrogen and containing no unsaturated bonds, having from one to eight carbon atoms and bound to the remainder of the molecule by a single

bond, e.g. methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, n-pentyl, etc. The alkyl radicals may optionally be substituted by one or more substituents chosen independently from the group comprising halogens, hydroxyl, alcoxides, carboxy, cyano, carbonyl, acyl, alkoxycarbonyl, amino, nitro, mercapto and alkylthio.

"alkoxy" refers to a radical of formula $-OR_a$, where R_a is an alkylo radical as described above, e.g. methoxy, ethoxy, propoxy, etc.

"alkoxycarbonyl" refers to a radical of formula $-C(O)OR_a$, where R_a is an alkylo radical as described above, e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, etc.

alkylthio" refers to a radical of formula -SRa, where Ra is an alkylo radical as described above, e.g. methylthio, ethylthio, propylthio, etc.

"amino" refers to a radical of formula -NH2

"aryl" refers to a phenyl or naphthyl radical. The aryl radical may optionally be substituted by one or more substituents selected from among the group comprising hydroxy, mercapto, halogens, alkyl, phenyl, alkoxy, haloalkyl, nitro, cyano, dialkylamino, aminoalkyl, acyl and alkoxycarbonyl as they are defined here.

"acyl" refers to a radical of formula $-C(O)-R_a$ and $-C(O)-R_b$, where R_a is an alkyl radical as described above and R_b is an aryl radical as described above, e.g. acetyl, propionyl, benzoyl, and similar.

"carboxy" refers to a radical of formula -C(O)OH

"cyano" refers to a radical of formula –CN

"cycloalkyl" refers to stable cycles of 3 to 10 monocyclic or bicyclic members that are saturated or partially saturated and consist exclusively of carbon and hydrogen atoms. This term also includes cycloalkylo radicals, which may optionally be substituted by one or more substituents chosen independently from the group comprising alkyl, halogen, hydroxy, amino, cyano, nitro, alkoxy, carboxy and alkoxycarbonyl

"halogens" refers to bromine, chlorine, iodine or fluorine

"haloalkyl" refers to an alkylo radical, as defined above, which is substituted by one or more halogens, also as defined above, e.g. trifluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, and similar

"heterocycle" refers to a heterocyclic radical. The heterocycle refers to a stable cycle of 3 to 15 members comprising carbon atoms and one to five heteroatoms chosen from the group comprising nitrogen, oxygen and sulphur. For the purposes of this invention, the heterocycle may be a monocyclic, bicyclic or tricyclic system that may include fused rings, and the nitrogen, carbon or sulphur atoms may optionally be oxidised, the nitrogen atom may optionally be quaternised, and the heterocycle may be partly or totally saturated or aromatic. Examples of these heterocycles include, but are not limited to, azepines, benzimidazol, benzothiazol, furan, isothiazol, imidazol, indol, piperidine, piperazine, purine, quinoline, thiadiazol, tetrahydrofuran. The heterocycle may optionally be substituted by R^3 and R^4 as defined in the summary of the invention.

"mercapto" refers to a radical of formula -SH

"nitro" refers to a radical of formula -NO2.

Preferred compounds

Examples of preferred compunds include those which conform with one or more of the following definitions:

X is CH_2;

N is 5 to 10, especially 6, 7, 8 or 9;

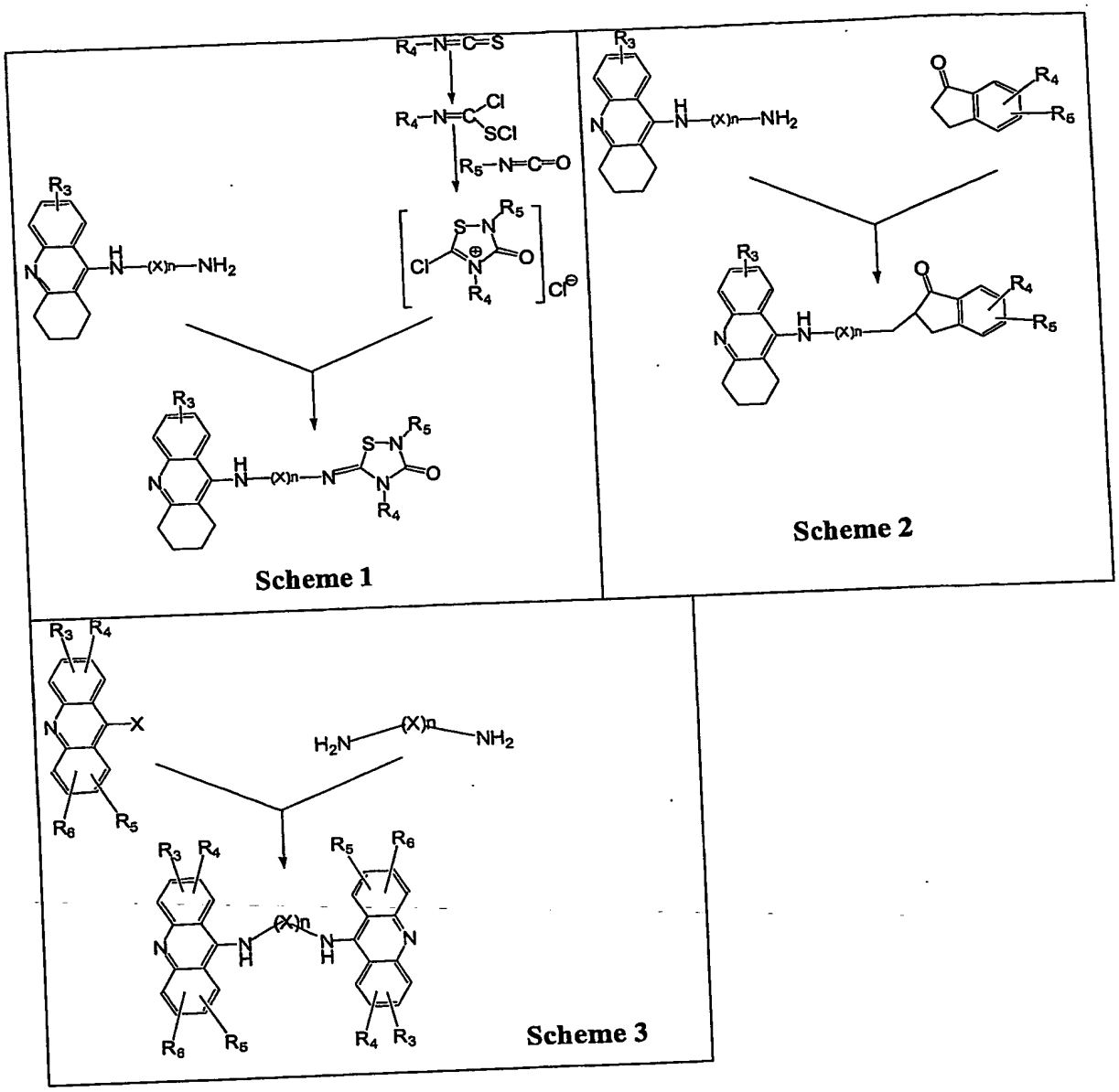
in formula (I), R_3 is hydrogen; R_4 and R_5 are alkoxy especially methoxy;

in formula (II), R_3 is hydrogen, R_4 is alkyl especially ethyl, R_5 is alkyl especially branched alkyl more especially isopropyl; alkoxycarbonylalkyl especially ethoxycarbonylmethyl;

in formula (III), R_3, R_4, R_5 and R_6 are hydrogen.

Synthesis of the compounds of the invention

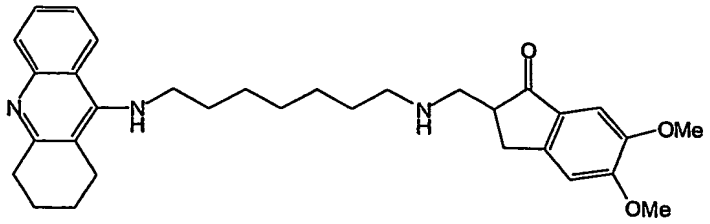
The synthesis of the compounds follow a convergent pathway strategy that could be summarized in schemes 1, 2 and 3.



9-alkylaminotetrahydroacridines has been synthesized following the procedure previously reported in bibliography. Carlier, P.R.; Chow. E.S.-H; Han, Y.; Liu, J.; El Yazal, J.; Pang Y.-P. *J. Med. Chem.*, **1999**,*42*, 4225-4231.

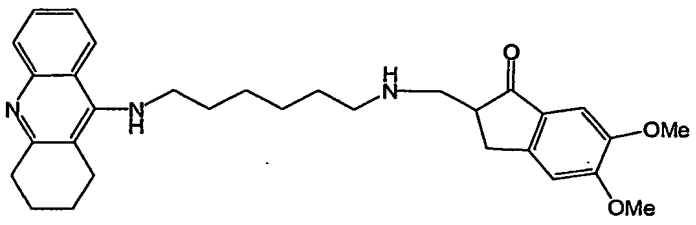
Specific examples are:

Example 1: 5,6-Dimethoxy-2-{[7-(1,2,3,4-tetrahydro-acridin-9-ylamino)-heptylamino]-methyl}-indan-1-one.



To a stirred solution of 9-(7-aminoheptylamino)- 1,2,3,4-tetrahydroacridine (134 mg, 0.43 mmol) in a mixture of ethanol: water 3:1 (3.5 ml) at room temperature, paraformaldehyde (26 mg, 0.86 mmol) and 5,6-dimethoxyindan-1-one (83 mg, 0.43 mmol) were added. The pH was adjusted to 3 with 35% hydrochloric acid and the mixture were refluxed for 24 hours. At the end of this period, the reaction mixture was cooled (25°C), the solvent was removal under vacuum pressure and the residue was treated with K_2CO_3 saturated solution (3.5 ml) and methylene chloride (5 ml). The organic layer was washed with water (5 ml) and dried (anhydrous Na_2SO_4). The solvent was removed under vacuum and the residue was purified by preparative centrifugal thin layer chromatography. Elution with 5: 1 ethyl acetate: methanol containing 1% of aquous ammonia afforded the title compound as yellow syrup (15 mg, 6.8 %). ^{1}H-RMN ($CDCl_3$, 300MHz, δ): 7.93 (dd, 2H, *J*=8.2 Hz), 7.53 (ddd, , 1H, *J*=8.2, 1.3 Hz), 7.32 (ddd, , 1H, *J*=8.2, 1.3 Hz), 7.13 (s, 1H), 6.85 (s, 1H), 3.94 (s, 3H), 3.88 (s, 3H), 3.48 (t, 2H, *J*=7.1 Hz), 3.28-3.19 (m, 1H), 3.10-3.05 (m, 2H), 2.89-2.56 (m, 9H), 1.91-1.88 (m, 4H), 1.63 (quint, 2H, *J*=7.5 Hz), 1.50-1.37 (m, 2H), 1.34-1.32 (m, 6H). ^{13}C-RMN ($CDCl_3$, 300MHz, δ): 203.0, 155.9, 151.3, 149.7, 149.6, 129.6, 128.8, 128.4, 123.9, 123.2, 107.6, 104.4, 56.5, 56.4, 51.5, 50.1, 49.7, 33.9, 31.9, 31.6, 30.0, 29.5, 27.4, 27.1, 27.0, 24.9, 23.2, 22.9. ESI-MS: m/z $[M+H^+]^+$ 516.

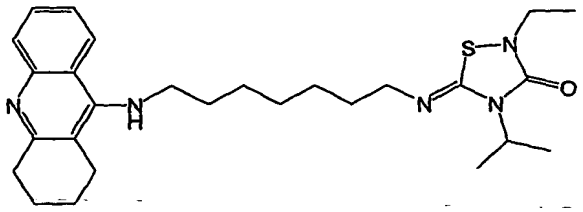
Example 2: 5,6-Dimethoxy-2-{[6-(1,2,3,4-tetrahydro-acridin-9-ylamino)-hexylamino]-methyl}-indan-1-one.



According to the general procedure in Example 1, 9-(6-aminohexylamino)-1,2,3,4-tetrahydroacridine (96 mg, 0.32 mmol), paraformaldehyde (19 mg, 0.64 mmol), 5,6-

dimethoxyindan-1-one (62 mg, 0.32 mmol) and 35% hydrochloric acid (pH=3) were refluxed for 24 hours. Purification by two preparative centrifugal thin layer chromatographies eluting with 10: 1 ethyl acetate: methanol containing 2% of aquous ammonia afforded the title compound as yellow syrup (8 mg, 5 %). ^{1}H-RMN ($CDCl_3$, 300MHz, δ): 7.97 (dd, 2H, *J*=8.1 Hz), 7.56 (ddd, , 1H, *J*=8.1, 1.2 Hz), 7.34 (ddd, , 1H, *J*=8.2, 1.2 Hz), 7.13 (s, 1H), 6.85 (s, 1H), 3.94 (s, 3H), 3.88 (s, 3H), 3.55 (t, 2H, *J*=7.0 Hz), 3.30-3.19 (m, 1H), 3.10-3.07 (m, 2H), 2.90-2.60 (m, 7H), 1.89-1.68 (m, 4H), 1.40-1.35 (m, 2H), 1.28-1.11 (m, 6H). ^{13}C-RMN ($CDCl_3$, 300MHz, δ): 207.1, 155.7, 151.2, 149.4, 149.3, 129.3, 128.7, 128.3, 123.8, 123.0, 107.4, 104.2, 56.3, 56.1, 51.3, 49.7, 49.4, 47.3, 31.6, 31.3, 29.7, 27.0, 24.6, 22.9, 22.5. ESI-MS: m/z $[M+H^+]^+$ 502.

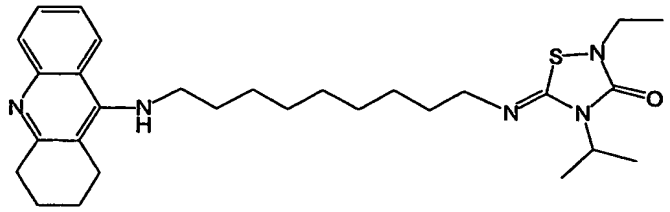
Example 3: 2-Ethyl-4-isopropyl-5-[7-(1,2,3,4-tetrahydro-acridin-9-ylamino)-heptyliminio]-[1,2,4]thiadiazolidin-3-one.



Clorine was bubbled slowly through a solution of ethylisothiocyanate (569 µl, 6.5 mmol) in dry hexane (15 ml) under nitrogen atmosphere at –15°C to –10°C. Chlorine was generated by addition of 35% HCl to $KMnO_4$. The temperature of the reaction mixture was carefully controlled during the addition step. At this point the N-ethyl-*S*-chloroisothiocarbamoyl chloride was formed. Afterward, isopropylisocyanate (640 µl, 6.5 mmol) was added. The mixture was stirred at room temperature during 10 hours and the solvent was evaporated to dryness. The residue was resolved in anhydrous tetrahydrofurane (510 ml) and 9-(7-aminoheptylamino)-1,2,3,4-tetrahydroacridine (105 mg, 0.34 mmol) and triethylamine (94 µl, 0.68 mmol) were added. The reaction mixture was stirred for 24 hours at room temperature, the white solid was filtered off, the solvent of solution was removed under vacuum and the residue was purified by silica gel flash chromatography. Elution with 4: 1 ethyl acetate: methanol containing 5% of aquous ammonia afforded the title compound as yellow syrup (24 mg, 15%). ^{1}H-RMN ($CDCl_3$, 300MHz, δ): 8.36 (d, 1H, *J*= 8.4 Hz), 8.09 (d, 1H, *J*= 8.4 Hz), 7.63 (t, 1H, *J*= 8.4 Hz), 7.39 (t, 1H, *J*= 8.4 Hz), 5.25 (s br, 1H), 4.58 (sept, 1H, *J*= 6.6 Hz), 3.80 (m, 2H), 3.73 (q, 2H, *J*= 7.0 Hz), 3.23 (t br, 2H, *J*= 5.9 Hz), 2.98 (t, 2H, *J*= 6.8 Hz), 2.60

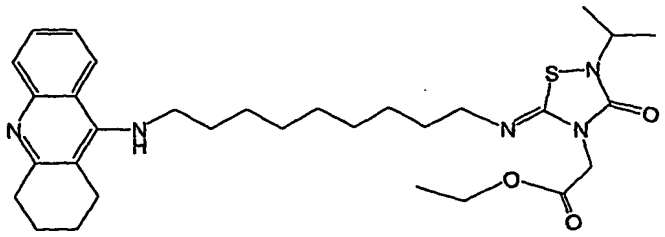
(t br, 2H, *J*= 6.8 Hz), 1.86-1.74 (m, 4H), 1.63 (t, 2H, *J*= 6.6 Hz), 1.39 (m, 4H), 1.21 (d, 6H, *J*=6.6 Hz), 1.20 (t, 3H, *J*= 7.0 Hz). ^{13}C-RMN ($CDCl_3$, 300MHz, δ): 160.2, 154.6, 146.9, 148.1, 139.1, 131.7, 128.1, 124.9, 123.9, 53.3, 48.8, 46.8, 38.2, 31.3, 30.6, 29.0, 27.2, 26.7, 23.7, 22.0, 21.0, 12.6. EI-MS: m/z $[M^+]^+$ 481.

Example 4: 2-Ethyl-4-isopropyl-5-[9-(1,2,3,4-tetrahydro-acridin-9-ylamino)-nonyliminio]-[1,2,4]thiadiazolidin-3-one.



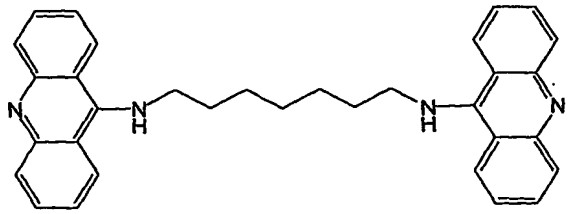
According to the general procedure in Example 3, by addition of chlorine to ethylisothiocyanate (569 µl, 6.5 mmol) N-ethyl-*S*-chloroisothiocarbamoyl chloride was formed and then isopropylisocyanate (640 µl, 6.5 mmol) was added. In the next step of the reaction, 9-(9-aminononylamino)-1,2,3,4-tetrahydroacridine (173 mg, 0.5 mmol), and triethylamine (140 µl, 1.0 mmol) were added and the mixture was stirred for 24 hours at room temperature. First purification by silica gel flash chromatography (15: 1 ethyl acetate: methanol and 2% of aquous ammonia) and second purification by preparative centrifugal thin layer chromatography (same eluent) afforded the title compound as yellow syrup (36 mg, 14 %). ^{1}H-RMN ($CDCl_3$, 300MHz, δ): 7.94 (dd, 1H, *J*=8.0, 0.5 Hz), 7.90 (d, 1H, *J*=8.8 Hz), 7.53 (ddd, , 1H, *J*=8.2, 7.1, 1.2 Hz), 7.32 (ddd, 1H, *J*=8.2, 7.1, 1.1 Hz), 4.59 (sept, 1H, *J*=6.6 Hz), 3.74 (q, 2H, *J*=7.1Hz), 3.48 (t, 2H, *J*=7.1 Hz), 3.01 (s br, 2H), 2.98 (t, 2H, *J*=7.1 Hz), 2.68 (s br, 2H), 1.91-1.88 (m, 4H), 1.66-1.59 (m, 4H), 1.29 (s br, 11H), 1.21 (d, 6H, *J*=6.6 Hz), 1.20 (t, 3H, *J*=7.1 Hz). ^{13}C-RMN ($CDCl_3$, 300MHz, δ): 158.5, 154.9, 148.5, 148.3, 139.5, 131.8, 128.7, 123.9, 123.1, 53.8, 49.8, 47.1, 38.5, 34.0, 32.0, 31.0, 29.7, 29.6, 27.6, 27.2, 25.0, 23.3, 22.9, 21.2, 12.9. EI-MS: m/z $[M^+]^+$ 509.

Example 5: {2-isopropyl-3-oxo-5-[9-(1,2,3,4-tetrahydro-acridin-9-ylamino)-nonyliminio]-[1,2,4]thiadiazolidin-4-yl}-acetic acid ethyl ester.



According to the general procedure in Example 3, by addition of chlorine to ethoxycarbonylmethylisothiocyanate (0.8 ml, 6.5 mmol) N-ethoxycarbonylmethyl -*S*-chloroisothiocarbamoyl chloride was formed and then isopropylisocyanate (640 µl, 6.5 mmol) was added. In the next step of the reaction, 9-(9-aminononylamino)-1,2,3,4-tetrahydroacridine (173 mg, 0.5 mmol), and triethylamine (140 µl, 1.0 mmol) were added and the mixture was stirred for 24 hours at room temperature. First purification by silica gel flash chromatography (15: 1 ethyl acetate: methanol and 2% of aquous ammonia) and second purification by preparative centrifugal thin layer chromatography (same eluent) afforded the title compound as yellow syrup (10 mg, 0.1 %). ^{1}H-RMN ($CDCl_3$, 300MHz, δ): 7.98 (d, 2H, *J*=7.8 Hz), 7.57 (t, 1H, *J*=7.6 Hz), 7.35 (t, 1H, *J*=7.6 Hz), 4.59 (sept, 1H, *J*=6.6 Hz),4.17 (q, 2H, *J*=7.1 Hz), 3.74 (q, 2H, *J*=7.1 Hz), 3.68-3.56 (m, 2H), 3.09 (s br, 2H), 2.98 (t, 2H, *J*=6.8 Hz), 2.65 (s br, 2H), 1.95-1.90 (m, 4H), 1.78-1.59 (m, 4H), 1.29-1.18 (m, 11H),1.21 (d, 6H, *J*=6.6 Hz), 1.20 (t, 3H, *J*=7.1 Hz). ^{13}C-RMN ($CDCl_3$, 300MHz, δ): 173.4, 151.6, 143.9, 148.3, 136.7, 131.8, 128.7, 123.6, 123.1, 77.2, 49.5, 42.3, 31.8, 30.7, 29.3, 26.9, 24.7, 23.0, 21.0, 14.1, 12.6. EI-MS: m/z $[M+H^+]^+$ 554.

Example 6: *N,N'*-di-acridin-9-yl-heptane-1,7-diamine.



To a stirred solution of 9-chloroacridine (200mg, 0.93 mmol) in 1-pentanol (5 ml), 1,7-heptanediamine (61 mg, 0.46 mmol) was added and the mixture was refluxed for 12 hours. At this time, the solid was filtered off and washed with acetonitrile to afford 170 mg (38 %) of the product as a yellow solid. ^{1}H-RMN ($CDCl_3$, 400MHz, δ): 8.34 (dd, 4H, *J*=8.4, 1.6 Hz), 7.73 (ddd, 4H, *J*=8.4, 6.8, 1.6 Hz), 7.53 (d, 4H, *J*=8.8 Hz), 7.29 (ddd, 4H, *J*=8.8, 6.8, 0.8 Hz), 2.92-2.88 (m, 4H), 1.7.-1.66 (m, 4H), 1.4 (s br, 8H). ^{13}C-RMN ($CDCl_3$, 400MHz, δ): 145.3, 137.6, 130.0, 125.3, 124.5, 121.0, 43.6, 32.9, 31.8, 30.0. ESI-MS: m/z $[M+H^+]^+$ 485.

Biological evaluation

AchE inhibition (from human erythrocytes)

The method of Ellman *et al.* (Ellman, G.L.; Courtney, K.D.; Andres, B.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88-95) was followed. The assay solution consisted of 0.1 M phosphate buffer pH 8, 200 μM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellam's reagent), 0.02 unit/ml AChE (Sigma Chemical Co. from human erythrocytes), and 400 μM acetylthiocholine iodide as the substrate of the enzymatic reaction. The compounds tested were added to the assay solution and pre incubated with the enzyme for 10 min at 30°C. After that period, the substrate was added. The absorbance changes at 412 nm were recorded for 5 min with a Perkin-Elmer 550 SE UV/VIS spectrometer, the reaction rates were compared, and the percent inhibition due to the presence of test compounds was calculated. The IC_{50} is defined as the concentration of each compound that reduces a 50% the enzymatic activity with respect to that without inhibitors.

Table 1: Human Erythrocytes AChE inhibition

Compound	IC_{50} (nM)
1	25
2	100
3	250
4	120
5	120

AChE inhibition (from bovine erythrocytes)

AChE inhibitory activity was evaluated at 25°C by the colorimetric method reported by Ellman (Ellman, G.L.; Courtney, K.D.; Andres, B.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88-95). The assay solution consisted on 0.02 unit/ml AChE from bovine erythrocytes, 0.1 M sodium phosphate buffer pH 8, 0.3 mM 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent), and 0.5 mM acetylthiocholine iodide as the substrate of the enzymatic reaction. Enzyme activity was determined by measuring the absorbance at 405 nm

during 10 minutes with a Fluostar optima plate reader (BMG). The tested compounds were preincubated with the enzyme for 10 minutes at 30°C.

In this conditions, compound **6** showed an IC_{50} value of 2.03 E-07 M.

Neuronal AChE activity

Acetylcholinesterase (AChE) enzyme preparations were obtained from SH-SY5Y, SK-N-SH and N2A cells.

CELL CUTURE: The human neuroblastoma cell line SH-SY5Y was cultured in minimum essential medium, Han's F12 medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and grown in a 5% CO_2 humidified incubator at 37°C.

The human neuroblastoma cell line SK-N-SH was cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and grown in a 5% CO_2 humidified incubator at 37°C.

The mouse neuroblastoma cell line N2A was cultured in DULBECCO'S MOD EAGLE medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, and grown in a 5% CO_2 humidified incubator at 37°C.

The cells were plated at 250 x 10^3 cells for each reaction, at least, 48 hours before the AChE activity measure. Cells were washed and harvested in 0.1 M sodium phosphate buffer pH 8, at 4°C.

INHIBITION OF AChE: AChE inhibitory activity was evaluated at 25°C by the colorimetric method reported by Ellman (Ellman, G.L.; Courtney, K.D.; Andres, B.; Featherstone, R.M. *Biochem. Pharmacol.* **1961**, *7*, 88-95). The assay solution consisted of AChE from neuronal cells, 0.1 M phosphate buffer pH 8, 0.3 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent), and 0.5 mM acetylthiocholine iodide as the substrate of the enzymatic reaction. Enzyme activity was determined by measuring the absorbance at 405 nm during 10 minutes with a Fluostar optima plate reader (BMG).The tested compounds were preincubated with the enzyme for 10 minutes at 30°C.

The reaction rate was calculated with, at least, triplicate measurements, and the percent inhibition due to the presence of test compound was calculated relative to the

compound-free control. The compound concentration producing 50% of AChE inhibition (IC_{50}) was determined.

		IC50 (M)		
		N2A (mouset neuroblastoma)	SK-N-SY (human neuroblastoma)	SH-SY5Y (human neuroblastoma)
AChE inhibitors	1	2,09E-07	1,09E-07	4,35E-06
	3	4,24E-07	4,13E-07	3,00E-07
	4	4,46E-07	4,41E-07	3,33E-07
	5	5,61E-07	7,78E-07	3,91E-07
	Tacrine	3,95E-07	3,03E-07	3,91E-07

Inhibition of β-amyloid aggregation:

The generation of AChE-Aβ complexes were carried out as described previously [39, 40]. Stock solutions of $A\beta_{1-40}$ at 3.5 mM were prepared in DMSO. The amount of peptide used in the assays was 0.1 mM. Human recombinant AChE (Sigma-Aldrich) was used at a molar ratio Aβ-AChE 200:1. For the aggregation studies the peptide was mixed with the appropriate amount of AChE in PBS pH 7.4 and stirred for 48 hours in a microtiter plate at room temperature. The fibrils obtained were characterized by Congo Red (CR) binding.

For the inhibition of β-amyloid aggregation, the compounds tested were used at the IC_{50} defined in the previous paragraph of the biological evaluation. Propidium iodide 50 μM for comparison. [42]

To quantify the amount of fibrils aggregated, the binding to CR was done as described by Klunk (Klunk, WE.; Pettegrew, JW.; Abraham, DJ. *J. Hystochem. Cytochem.*, **1989**, 8, 1293-1297). Briefly, 5.5 µl aliquot of the aggregation mixture were added to 132 µl of a solution of 25 µM CR (100 mM phosphate buffer pH 7.4, 150 mM NaCl) and incubated for 30 minutes at room temperature. Absorbance was measured at 480 and 540 nm. The CR binding was estimated by CR (M)= $(A_{540}/25295)-(A_{480}/46306)$.

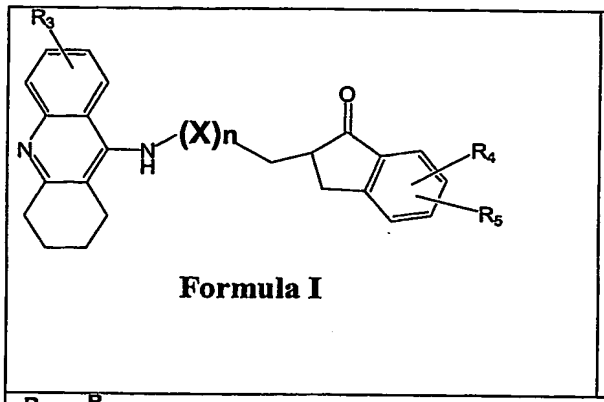
In the conditions above described, the indanone-tacrine derivative **1** showed a 18.7% reduction of the β–amyloid-AChE complex aggregation, while the thiadiazolidinone-tacrine derivative **5** decrease the β-amyloid-AChE complex aggregation by 27.8%. The peripheral inhibitor propidium reduces the aggregation of the β-amyloid-AChE complex by 18.1 %. This compound was used as standard of reference.

Pharmaceutical compositions and methods of treatment

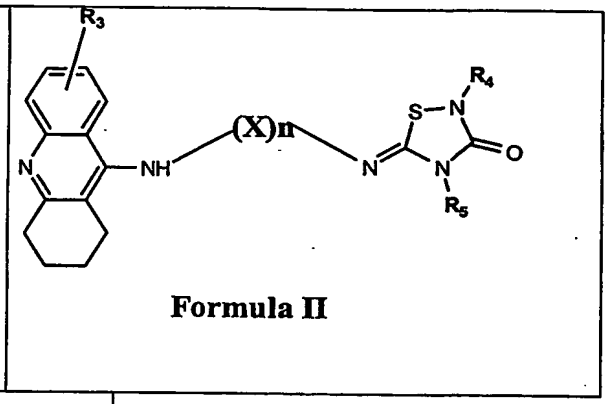
Having regard to the above examples, the present invention also provides pharmaceutical compositions which comprise a compound of this invention and a pharmaceutically acceptable carrier. The compounds and compositions of this invention can be employed in a method of treating Alzheimer's disease, cognitive disorders and/or neurodegenerative dementing disease with aberrant protein aggregations. The invention also provides for the use of the compounds of this invention in the preparation of pharmaceutical compositions for treatment Alzheimer's disease, cognitive disorders and/or neurodegenerative dementing disease with aberrant protein aggregations.

Claim

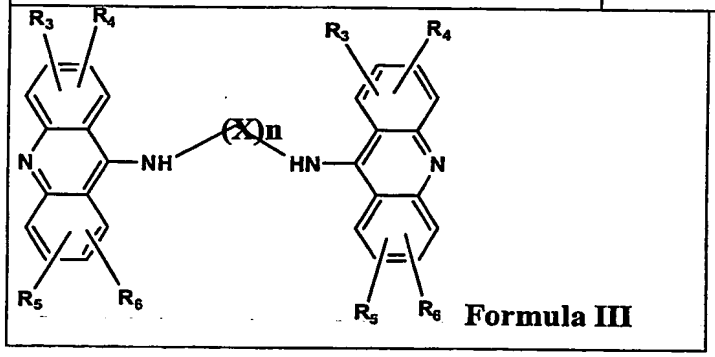
A compound represented by the general formula I, II or III:



Formula I



Formula II



Formula III

where:

X	is $-C(R^1)(R^2)-$, -CO-, -O- or $-NR^1-$
n	is zero, one, two, three, four, five, six, seven, eight, nine or ten
R^1 and R^2	are independently selected from hydrogen, alkyl, aryl, halo, haloalkyl
R^3, R^4, R^5 and R^6	are independently selected from hydrogen, alkyl, cycloaklyl, haloalkyl, halo, aryl, $-(Z)_n$-aryl, heteroaryl, $-OR^3$, $-C(O)R^3$, $-C(O)OR^3$, $-S(O)_t-$
t	is zero, one or two
Z	is independently selected from $C(R^3)(R^4)-$, -C(O)-, -O-, $-C(=NR^3)-$, $-S(O)_t-$, $N(R^3)-$.